Registration of 'CP 04-1566' Sugarcane

Jack C. Comstock, Barry Glaz,* Serge J. Edmé, R. Wayne Davidson, Robert A. Gilbert, Neil C. Glynn, Duli Zhao, Sushma Sood, Jimmy D. Miller, and Peter Y. P. Tai

ABSTRACT

'CP 04-1566' (Reg. No. CV-152, PI 667622) sugarcane (a complex hybrid of *Saccharum* spp.) was developed through cooperative research conducted by the USDA-ARS, the University of Florida, and the Florida Sugar Cane League, Inc., and was released to growers in Florida on 30 Sept. 2011. CP 04-1566 was selected from the cross X01-0246 ('CP 89-2377' × 'CP 96-1252') made at Canal Point on 29 Nov. 2001. Both parents were released for commercial production: CP 89-2377 for organic (muck) soils and CP 96-1252 for both muck and sand soils. CP 04-1566 was tested in stage 4 on sand soils in Florida because of its superior yields on sand soils in stage 3. CP 04-1566 was released for sand soils because of its resistance to all the major diseases in Florida: brown rust (caused by *Puccinia melanocephala* H. & P. Sydow) even though it does not contain the gene for brown rust resistance (*Bru*1), orange rust (caused by *P. kuehnii* E.J. Butler), *Sugarcane mosaic virus* strain E (mosaic), smut (caused by *Ustilago scitaminea* H. & P. Sydow), and ratoon stunt (caused by *Leifsonia xyli* subsp. *xyli* Evtushenko et al.), and it is resistant to leaf scald (caused by *Xanthomonas albilineans* Ashby, Dowson), in Florida. CP 04-1566 has a cane yield and commercial recoverable sucrose (CRS) equal to those of the commercial check, 'CP 78-1628'. CP 04-1566 is susceptible to *Sugarcane yellow leaf virus* and had moderate to poor tolerance to freezes on the basis of its rank in regard to CRS in 2010–11 and 2011–12 at the University of Florida Hague Farm, near Gainesville, FL..

'CP 04-1566' (Reg. No. CV-152, PI 667622) is a sugarcane (a complex hybrid of *Saccharum* spp.) derivative of a long-term recurrent selection program conducted through a tripartite cooperative research program of the USDA-ARS, the University of Florida, and the Florida Sugar Cane League, Inc. It was released in Florida on 30 Sept. 2011. Modern sugarcane cultivars, such as CP 04-1566, are allopolyploid (with aneuploidy) hybrids, and in the mainland USA, they can be traced back to 17 initial ancestral clones (Deren, 1995) derived from *Saccharum officinarum* L. These ancestral clones were used to make crosses with *Saccharum spontaneum* L. clones, and the F_1 hybrids were backcrossed to the *S. officinarum* background to recover the trait for high sucrose content (Roach, 1972; Sreenivasan et al., 1987). Modern sugarcane cultivars represent advanced generations of long-term breeding that began with these backcrosses.

J.C. Comstock, B. Glaz, S.J. Edmé, N.C. Glynn, D. Zhao, S. Sood, J.D. Miller (retired), and P.Y.P. Tai (deceased), USDA-ARS Sugarcane Field Stn., 12990 U.S. Highway 441 N, Canal Point, FL 33438; R.W. Davidson, Florida Sugar Cane League, Inc., P.O. Box 1208, Clewiston, FL 33440; R.A. Gilbert, Univ. of Florida, Everglades Res. and Educ. Ctr., 3200 East Palm Beach Road, Belle Glade, FL 33430. *Corresponding author (Barry.Glaz@ars.usda.gov). Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by USDA, the University of Florida, or the Florida Sugar Cane League, Inc.

Abbreviations: CP, Canal Point; CRS, commercial recoverable sucrose; RCBD, randomized complete block design; SCYLV, *Sugarcane yellow leaf virus*.

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CP 04-1566 was tested only on sand soils in stage 4 because of its superior yields on sand soils and substandard yields on organic soils in stage-3 trials in Florida. CP 04-1566 was released because of its resistance to brown rust (caused by *Puccinia melanocephala* H. & P. Sydow) even though it does not have the *Bru*1 gene, a major gene for brown rust resistance (Glynn et al., 2013), to orange rust (caused by *Puccinia kuehnii* E.J. Butler), to smut (caused by *Ustilago scitaminea* H. & P. Sydow), to ratoon stunt (caused by *Leifsonia xyli* subsp. *xyli* Evtushenko et al.), and to *Sugarcane mosaic virus* strain E (mosaic); because it is resistant to leaf scald (caused by *Xanthomonas albilineans* Ashby, Dowson) in Florida; and because of its acceptable yields and levels of commercial recoverable sucrose (CRS). The name CP 04-1566 was assigned according to the routine Canal Point (CP) naming protocol, being the 566th selection assigned in the year 2004 in the first clonal selection stage. Selection numbers ranging from 1000 to 2999 are reserved for genotypes selected from the CP sugarcane cultivar breeding and selection program (CP program) that are bred for the Florida industry.

CP 04-1566 was selected from the cross X01-0246 ('CP 89-2377' [PI 607919] × 'CP 96-1252' [PI 634935]) made at Canal Point, FL on 29 Nov. 2001. Both parents had been released for commercial production: CP 89-2377 on organic soils only and CP 96-1252 for both organic and sand soils. CP 89-2377, the female parent (Miller et al., 2000), had 7% higher sugar yields on organic soils in the final selection than the commercial check, ‘CP 70-1133’ (MIA 34310; Rice et al., 1978). The male parent, CP 96-1252, had both high cane yields and high CRS levels on organic and sand soils (Edmé et al., 2005). Only one grandparent of CP 04-1566 is known, NG 77-252, the maternal parent of CP 96-1252 (Edmé et al., 2005).

Methods

Early Selection Stages

CP 04-1566 was selected through standard selection procedures of the CP program as described by Tai and Miller (1989). The cross (X01-0246) CP 89-2377 × CP 96-1252 was made at Canal Point on 20 Dec. 2001 (Table 1). The F_1 seeds of this cross were planted in flats in a greenhouse early in 2003, and germinated plants were transplanted to the field in May 2003 at Canal Point. Approximately 50,000 other genotypes were also in the seedling stage that was planted in May 2003. From this stage on, the CP program propagated genotypes clonally. One stalk from the stool that was to become CP 04-1566 was selected from the seedling stage and advanced to stage 1 in January 2004 with about 15,000 other unreplicated selections. Stage-1 plots each consisted of one row that was 0.5 m long, and they were separated by 0.5-m alleys. As in all other selection stages, the row spacing was 1.5 m. Selection in seedling stage and stage 1 was visual. Emphasis was placed on vigor and resistance to natural infection of brown rust, smut, and leaf scald.

CP 04-1566 was planted in stage 2 at Canal Point in November 2004 with 1486 other unreplicated genotypes advanced from stage 1. Stage-2 plots consisted of two rows that were 4.5 m in length. Plots were arranged in sections, two plots in length, such that the back end of the first plot was separated from the front end of the second plot by a 1.5-m alley. The back end of the second plot in each section was separated from the front end of the first plot of the next section by a 6.0-m alley. Thus, plots within each section were separated by 1.5-m alleys to ensure delineation of plots, and sections were separated by 6.0-m alleys to allow for vehicle access for sampling and harvesting. Cultivar CP 89-2143 (PI 607918; Glaz et al., 2000a) was the primary reference cultivar in stage 2, and it was replicated 13 times. Visual ratings were made in stage 2 on growth habit and agronomic traits. Genotypes that were highly recumbent, had protruding buds, had many broken stalks, and generally appeared unsuitable for commercial production were discarded. Genotypes with symptoms of leaf scald, smut, brown rust, and any other disease symptoms were also not selected.

Stalks were counted in stage 2 in July and August 2005. In October 2005, 10-stalk samples were collected from each plot and weighed. Cane yield (*C*), was calculated as the product of stalk weight by stalk number:

$$C\ (\mathrm{Mg\ ha^{-1}}) = \text{stalk weight}\ (\mathrm{kg\ stalk^{-1}}) \times \text{stalk number}\ (\mathrm{stalks\ ha^{-1}}) \div 1000$$

All 10-stalk samples were then milled to extract juice and determine theoretical recoverable sucrose content, which was calculated as described by Legendre (1992). Fiber in this formula was estimated as 10% for all genotypes in stages 2 and 3 and estimated as described later in stage 4. All values of theoretical recoverable sucrose were multiplied by 0.86 to approximate CRS. Similarly, Legendre (1992) reported the calculation of a liquidation factor (ranging from 0.83 to 0.90) that was used by commercial mills in Louisiana to convert theoretical recoverable sucrose to CRS. Theoretical economic index (profitability) was calculated with a procedure that integrated sucrose content with costs of harvesting, hauling, and milling the cane in Florida (Deren et al., 1995).

The principal selection criteria in stage 2 (and later in stages 3 and 4) were CRS, profitability, sucrose yield, and resistance to diseases (primarily brown rust, mosaic, and leaf scald). Selection for resistance to orange rust was also conducted after the disease was introduced in 2007 (Comstock et al., 2008). Sucrose yield (*S*) was calculated as

$$S\ (\mathrm{Mg\ ha^{-1}}) = C\ (\mathrm{Mg\ ha^{-1}}) \times \mathrm{CRS}\ (\mathrm{kg\ Mg^{-1}}) \div 1000$$

Yield Trials in Commercial Fields

From stage 2, 135 genotypes were advanced to stage 3 in November and December 2005. Stage-3 genotypes and three reference cultivars—CP 72-2086 (CSR 458; Miller et al., 1984), CP 78-1628 (PI 542105; Tai et al., 1991), and CP 89-2143—were planted in yield trials in commercial fields at four growers’ farms representative of the Florida sugarcane industry. The farms of A. Duda & Sons, Inc., Okeelanta Corporation, and Sugar Farms Cooperative North–Osceola Region had organic (muck) soils, and Hilliard Brothers of Florida, Ltd. (Hilliard) had a sand

Table 1. Summary of the process leading to the release of sugarcane cultivar CP 04-1566 in Florida.

Year	Month	Stage and selection decision	Genotypes in stage	Locations
2001	December	Cross made at USDA-ARS Sugarcane Field Station	—	Canal Point, FL
2003	May	Germinated true seed transplanted into field (Seedlings)	50,000	Canal Point, FL
2004	January	Advanced from plant-cane seedlings to Stage 1	15,000	Canal Point, FL
2004	September	Assigned name CP 04-1566 in stage 1	15,000	Canal Point, FL
2004	November	Advanced from plant-cane stage 1 to stage 2	1,486	Canal Point, FL
2005	November–December	Advanced from plant-cane stage 2 to stage 3	135	Four farms in Florida
2007	November–December	Advanced from first-ratoon stage 3 to stage 4 sand soils	13	Two farms in Florida
2012	September	Cultivar release	1	—

soil. All four trials had two replications of each genotype planted in randomized complete block designs (RCBDs) in plots with two rows 4.5 m long. Plots were arranged in sections such that the first plot was separated from the second by a 1.5-m alley. The second plot in each section was separated from the first plot of the next section by a 6.0-m alley. Data were collected in the plant-cane (October 2006 and January 2007) and first-ratoon (October 2007) crops. Estimates of cane and sucrose yields and profitability were determined as described for stage 2. Because of its cane and sucrose yields and profitability on the sand soils at Hilliard and its moderate resistance to brown rust and orange rust (Comstock et al., 2008) by natural infection and to leaf scald and mosaic by artificial inoculation and natural infection, CP 04-1566 was among 13 genotypes selected for advancement from stage 3 to stage 4 on sand soils in November 2007. CP 04-1566 was not advanced to muck soils because of unacceptable yields.

The 13 stage-4 genotypes selected for sand soils, including CP 04-1566, were planted in yield trials in 2007 within commercial fields with sand soils at three growers' farms: 6 December at Hilliard, 3 November at Lykes Brothers, Inc., and 16 November at USSC. The primary reference cultivar was CP 78-1628, but the reference cultivars CP 72-2086 and CP 89-2143 were also included in these trials. All trials had six replications with genotypes planted in RCBDs in plots three rows wide and 10.5 m long. Alleys of 1.5 m separated plots. Experiments were generally 2 plots wide and 48 plots long. Cane tonnage was estimated by first counting stalks in the two interior rows of each plot from July through September in 2008 (plant cane), 2009 (first ratoon), and 2010 (second ratoon). Stalk weight and CRS were estimated as described for stage 2 from a 10-stalk sample collected from the middle row of each plot on 13, 27, and 29 Jan. 2009 (plant cane), 8 and 16 Dec. 2009, and 26 Jan. 2010 (first ratoon) and 27 Sept. 2010 and 26 and 27 Oct. 2010 (second ratoon).

Seventeen samples of CP 04-1566 were processed for analysis of fiber content. Each sample consisted of five stalks and was collected from a border row (so as not to affect rows used for yield estimation). Leaves were stripped from these stalks, which were then cut into three approximately even sections (bottom, middle, and top stalk sections). Two randomly selected bottom, middle, and top sections were processed through a Jeffco cutter-grinder (Jeffries Brothers, Ltd.). About 400 g of shredded cane were collected and weighed. Brix values were measured with a hand refractometer on juice that had been extracted from the shredded cane by pressing it at 69 MPa for 30 s. The pressed samples were then weighed, crumbled, placed in paper bags, and dried at 60°C to a constant weight. Fiber percentages were then measured as described by Tanimoto (1964). Samples of a reference cultivar were processed on all dates when fiber samples of CP 04-1566 were processed. All fiber percentages calculated on a given day were corrected to the historic fiber content of the reference cultivar. For example, the reported fiber content of CP 78-1628 was 103.9 g fiber kg^{-1} cane (Tai et al., 1991). On days when CP 78-1628 was the reference cultivar, if its estimated fiber was 100.0 g kg^{-1}, then all estimated fiber samples of other genotypes were multiplied by 1.039. The corrected value for fiber content of CP 04-1566 was used in the formula reported by Legendre (1992) for calculating CRS.

Agronomic and Botanical Descriptions

Data for the agronomic and botanical descriptions of CP 04-1566 were recorded on 10 representative stalks sampled on 18 Aug. 2011 from a field with a Malabar sand soil at Townsite near Clewiston, FL. Stalks were sampled from the inner rows, and the agronomic and botanical descriptions were based on Artschwager and Brandes (1958). Colors were characterized according to Munsell Color Charts for Plant Tissues (Munsell Color Company, 1977). Stalks of CP 04-1566 were compared with those of CP 78-1628 when both cultivars were 288 d after planting for these descriptions.

Molecular Characterization of CP 04-1566

Six pairs of microsatellite primers (Table 2) developed through the International Consortium for Sugarcane Biotechnology (Cordeiro et al., 2003) were used to generate a genetic fingerprint for CP 04-1566. This was compared with those of cultivars CP 72-2086, CP 78-1628, CP 80-1743 (PI 542104; Deren et al., 1991), CP 84-1198 (PI 578049; Glaz et al., 1994), and CP 89-2143. Polymerase chain reaction conditions were as previously described (Glynn et al., 2009) with the following modifications: thermocycling at 95°C for 3 min, 94°C for 45 s, six cycles of 68°C for 5 min (decreasing by 2°C per cycle), 72°C for 1 min, 94°C for 45 s, eight cycles of 58°C for 2 min (decreasing by 1°C per cycle), 72°C for 30 s, and 24 cycles of 94°C for 45 s, 50°C for 2 min, and 72°C for 30 s followed by a final extension of 72°C for 7 min. CP 04-1566 was also tested for *Bru*1, a major gene for resistance to brown rust of sugarcane.

Disease Screening of CP 04-1566

Disease screening of CPCL 04-1566 was conducted by inoculation tests and/or by monitoring for natural infection to brown and orange rust, *Sugarcane yellow leaf virus* (SCYLV), smut, mosaic, and leaf scald.

Table 2. Size range and number of fragments generated by each of six microsatellite primer pairs from sugarcane cultivars CP 72-2086, CP 78-1628, CP 80-1743, CP 84-1198, CP 89-2143, and CP 04-1566.

		Number of fragments		
			From CP 04-1566	
Primer name	Size range of fragments	Total (all six cultivars)	Total	Unique
	bp			
SMC222CG	165–211	4	2	1
SMC221MS	122–144	4	2	0
SMC179SA	115–219	13	6	1
SMC1493CL	105–155	11	6	0
mSSCIR14	221–256	6	4	0
mSSCIR53	178–244	6	3	1

Rust

Screening for brown and orange rust was based on natural infection. The rating scale of rust infection responses in these evaluations consisted of five classes: 0 (resistant), 1 (moderately resistant), 2 (moderately susceptible), 3 (susceptible), and 4 (highly susceptible), which were determined primarily on the bases of size and number of uredia.

Yellow Leaf

To assay for the presence of SCYLV, leaf samples were collected from the sugarcane plants and preserved for the day in plastic bags. On the same day, tissue prints of the leaf midribs were made on nitrocellulose membranes, which were developed serologically.

Mosaic Ratings

Screening for mosaic was conducted in 2007, 2008, and 2009. Single bud cuttings were planted in flats (two replications with 30 cuttings each) and grown in the greenhouse. When the plants were 15 cm tall, they were inoculated with a painters air brush attachment at 551.6 kPa and a suspension of sap containing *Sugarcane mosaic virus* strain E that had been freshly prepared by grinding symptomatic leaves of sorghum 1 wk after inoculation. Four to 6 wk after inoculation, the mosaic infection was determined and an ANOVA was conducted and compared with that from CP 72-2086.

Leaf Scald Ratings

Similarly, for leaf scald screening, single bud cuttings of sugarcane were inoculated by spraying the freshly cut ends of bud cuttings with 10^8 cells mL^{-1} of *Xanthomonas albilineans* that was suspended from 6-d cultures using a painters air brush attachment at 275.8 kPa. For each clone there were three replications, each having 30 inoculated cuttings that were planted in a flat and grown in the greenhouse for 10 to 12 wk for symptom development. A moderately susceptible check, CP 80-1743, which is commercially grown on 25% of the acreage in Florida, was also inoculated.

Smut Ratings

Reaction to sugarcane smut disease was evaluated in replicated inoculated tests using a standard inoculation procedure that consisted of immersing five sugarcane cuttings (three buds per cutting) per clone in a suspension of 10^6 spores mL^{-1} for 30 min, incubated overnight under a plastic tarp, and planted the next day. Four replicate plots 5 m long were planted. The number of infected stalks per plot was determined. Clones more susceptible than the check CP 78-1628 were evaluated in natural infection tests. CP 78-1628 shows a moderately susceptible reaction to smut in inoculated tests but has been grown successfully commercially with few or no symptoms of smut and no yield losses.

Ratoon Stunt Ratings

Field inoculation tests of ratoon stunt disease were conducted in 2005, 2007, and 2008. A single stalk of each clone was inoculated at planting by cutting the stalk with a machete that had been immersed in juice crushed from infected stalks of the highly susceptible cultivar, CP 53-1, which supports high populations of ratoon-stunting bacteria. Stalks of a disease-free susceptible check, 'CP 72-1210' (MIA 34313; Miller et al., 1981), and a resistant check, CP 72-2086, were also inoculated. At 10 to 12 mo after inoculation, each clone was sampled by taking a section approximately 25 cm long from five stalks per plot at the base of the plant next to the soil. The number of plots for the stages were: stage 1 (1500 clones) a single plot, stage 3 (135 clones) two plots, stage 3 increase (40 clones) four plots and stage 4 (18–20 clones) four plots. One-cm diameter cores of internodal stalk tissue were taken and imprinted onto nitrocellulose membrane, and the number of bacterial colonized vascular bundles was determined with a tissue blot immunoassay (Comstock et al., 2001).

Statistical Analyses

Analyses of the stage-4 tests were done using PROC MIXED of SAS (SAS Institute, 2003). Data were analyzed for each crop cycle separately and for the combined plant-cane, first-ratoon, and second-ratoon crops. Within-year analyses used a mixed model with genotypes considered as fixed effects and locations and replications within locations considered as random effects. Across-year analyses used a mixed model with genotypes and crop cycles as fixed effects and locations and replications within locations considered as random effects. Differences among genotypes for cane yield, CRS, sucrose yield, and economic index were declared significant on the basis of the Student's paired *t*-test procedure at $P = 0.05$.

Characteristics

Field Performance

CP 04-1566 was tested in nine harvests at three trial locations in Florida during the 2008–2009 (two plant-cane harvests), 2009–2010 (two first-ratoon harvests), and 2010–2011 (2 second-ratoon harvests) seasons. The fiber content of CP 04-1566 was 97.3 g kg^{-1}. Stalks of CP 04-1566 weighed slightly less than CP 78-1628 in the plant crop but had similar weights in the first- and second-ratoon crop cycles (Table 3).

The CRS values of CP 04-1566 was significantly higher in the first-ratoon crop than CP 78-1628, the reference cultivar for sand soils, but did not differ in the plant and second-ratoon crops (Table 3). However, the cane yields, sucrose yields, and economic indices of CP 04-1566 were significantly greater than those of CP 78-1628 in the first-ratoon crop but did not differ in the other crops. Although not significant in all crops in the crop cycle, the three-crop mean economic index of CP 04-1566 was \$312 ha^{-1} greater than that of CP 78-1628.

In the CP sugarcane cultivar development program in Florida, decisions to advance and commercially release genotypes in the final three selection stages are made by a committee composed of sugarcane farmers and scientists from the public and private sectors. Members of this committee recommended releasing CP 04-1566 on 1 June

Table 3. Plant-cane, first-ratoon, and second-ratoon crop stalk weights, cane yields, commercial recoverable sucrose values, sucrose yields, and economic indices of CP 04-1566 and reference cultivar CP 78-1628 planted on sand soils at three locations.

	Crop cycle			
Cultivar	Plant cane	First ratoon	Second ratoon	Mean
	Stalk weight (kg)			
CP 04-1566	1.3	0.9	0.8	1.0
CP 78-1628	1.5	0.8	0.8	1.0
$p > t$	<0.01	ns	ns	ns
	Cane yield (Mg ha^{-1})			
CP 04-1566	154.8	95.3	94.7	114.9
CP 78-1628	141.5	78.8	91.5	104.0
$p > t$	0.11	0.01	ns	ns
	Commercial recoverable sucrose (g kg^{-1})			
CP 04-1566	123.9	126.3	112.8	125.0
CP 78-1628	127.1	122.6	113.7	124.8
$p > t$	ns	0.02	ns	ns
	Sucrose yield (Mg ha^{-1})			
CP 04-1566	21.1	11.9	10.8	14.6
CP 78-1628	19.5	9.5	10.3	13.1
$p > t$	ns	0.01	ns	ns
	Economic index ($ ha^{-1})			
CP 04-1566	4394	2094	1520	2670
CP 78-1628	4048	1573	1450	2358
$p > t$	ns	0.01	ns	ns
Locations	3	3	3	

2011 because of its resistance or moderate resistance to all major and minor sugarcane diseases found in Florida (except for *Sugarcane yellow leaf*) and its yields of cane and sucrose, which were equal to those of the reference check CP 78-1628 on sand soils.

Agronomic, Botanical, and Molecular Descriptions

The stalk heights, which were measured from the ground to the top visible dewlap, were 101 cm for CP 04-1566 and 108 cm for CP 78-1628 (Table 4). The color of the exposed stalks were 2.5 GY 6/4 for CP 04-1566 and 5GY 6/6 for CP 78-1628. The mean internode length of CP 04-1566 was 4.0 cm less than that of CP 78-1628. Internode shape was cylindrical for CP 04-1566 and conoidal for CP 78-1628. Growth cracks were few and of moderate depth on both CP 04-1566 and CP 78-1628.

The stalk diameter on each cultivar was measured at the middle of the 2nd, 5th and 10th internodes from the ground and from the uppermost hardened internode. The diameter of CP 78-1628 was 0.8 mm larger than that of CP 04-1566 at the 2nd internode from the ground (Table 4). In contrast the diameters of CP 78-1628 at the 5th and 10th internodes were smaller by 2.8 and 2.5 mm than CP04-1566. The mean width of the root bands, which was measured at the 5th and 10th internodes from the ground, was similar for each cultivar. Neither CP 04-1566 nor CP 78-1628 had bud furrows.

The shape of CP 04-1566 buds was round with a central germ pore compared with ovate with an emarginated basal wing region for CP 78-1628 (Table 4). The color of the buds on both cultivars was similar: a shade of green yellow, 5Y 8/6. The bud length and width of CP 04-1566 were 9.1 mm and 9.6 mm, compared with a length of 8.5 mm and a width of 7.2 mm for CP 78-1628.

The leaf shape of each cultivar was erect with a drooping tip, ascending (Table 4). The mean leaf blade length at the top visible dewlap was smaller for CP 04-1566 (156.5 cm) than for CP 78-1628 (187.7 cm). However, the leaf of CP 04-1566 (4.6 cm) was wider than the leaf of CP 78-1628 (4.0 cm). The leaf sheaths of CP 04-1566 and CP 78-1628 were loose on the stalks. Both cultivars had light pubescence on the center of their leaf sheaths. The midrib width of CP 04-1566 (5.1 mm) was 0.4 mm less than that of CP 78-1628 (5.5 mm). Midrib color on the adaxial leaf side was white for both cultivars, and the midrib color on the abaxial leaf side was green yellow for CP 04-1566 (5 GY 5/6) and yellow for CP 78-1628 (7.5Y 6/4).

Auricles were absent from CP 04-1566 (Table 4). The auricles on CP 78-1628 were short (4.7 mm), deltoid, and absent on the opposite side. The fourth dewlap below the top visible dewlap was squarish deltoid on CP 04-1566, and it was green-yellow (5Y 5/4). The corresponding dewlap of CP 78-1628 was deltoid and yellow (5Y 5/2) with a wax cover. The shape of the ligule on each cultivar was crescent with lozenge. The ligule color of each cultivar was a shade of yellow: 2.5GY 7/2 for CP 04-1566 and 5Y 7/2 for CP 78-1628.

Molecular Characterization of CP 04-1566

The six microsatellite primer pairs amplified 23 fragments, ranging from 105 to 256 bp, in CP 04-1566 (Table 2). The number of fragments amplified by each primer pair ranged from 2 to 6. Of the 23 fragments amplified, 16 were polymorphic and 7 monomorphic among the six genotypes. CP 04-1566 shared 17 fragments with CP 72-2086, 17 with CP 78-1628, 14 with CP 80-1743, 14 with CP 84-1198 and 13 with CP 89-2143. Three fragments were unique to CP 04-1566 among the six genotypes tested. These were identified in the fingerprints obtained using primer pairs SMC222CG (211 bp), SMC179SA (148 bp), and mSSCIR53 (191 bp). *Bru*1 was not detected in the DNA of CP 04-1566.

Disease Reactions

On the basis of screening in inoculated tests and on natural infection in field plots, CP 04-1566 is moderately resistant to resistant to the following diseases: brown rust, orange rust, mosaic, smut, and ratoon stunt and is resistant to leaf scald in Florida (Table 5). Because of the natural infection symptoms observed, CP 04-1566 was classified as susceptible to SCYLV, as are most other CP genotypes and commercial sugarcane cultivars in Florida. Flynn et al. (2005) suggested that losses in sucrose yield due to SCYLV ranged from −3.4 to 8.0% in Florida.

Table 4. Botanical descriptions of sugarcane cultivar CP 04-1566 and reference cultivar CP 78-1628 as measured in field plantings on a sand soil Townsite Farm of United States Sugar Corp. near Clewiston, FL.

Trait†	CP 04-1566	CP 78-1628
Stalk height (cm)	101	108
Stalk diameter (mm):		
Low, 2nd internode	28.4	29.2
Middle, 5th internode	27.5	24.7
Upper, 10th internode	25.3	22.8
Leaf shape	Erect with drooping tip, ascending	Erect with drooping tip, ascending
Leaf sheath pubescence	Light down center of sheath, moderate on younger leaves	Light down middle of sheath
Leaf length (cm)	156.5	187.7
Leaf width (cm)	4.6	4.0
Leaf midrib width (mm)	5.1	5.5
Stalk bud shape	Round with central germ pore, significant wings extend halfway down side	Ovate with emarginated basal wing region
Stalk bud length (mm)	9.1	8.5
Stalk bud width (mm)	9.6	7.2
Short auricle shape and length (mm)	Absent	Mostly present
Long auricle shape and length (mm)	Absent	Deltoid; 4.7
Internode shape	Cylindrical	Conoidal
Internode length (cm)	15.7	19.7
Growth cracks	Few medium depth	Few moderate depth
Bud furrows	None	None
Root band width (mm)	7.0	7.0
Growth ring width (mm)	3.2	2.5
Dewlap (leaf collar) shape	Squarish deltoid	Deltoid
Ligule shape	Crescent with lozenge	Crescent with broad lozenge

†Traits were measured on the fifth internode from the ground. Stalk and leaf traits are means of ten measurements. Stalk diameter was measured at the second internode from the ground (low), the fifth internode from the ground (middle), and at the hardened internode closest to the top visible dewlap (upper). Leaf length and width were measured from top most dewlap leaves. Internode lengths are means of the fifth internode from the bottom of ten stalks. Measurements were taken on 16 Aug. 2011, 369 d after planting.

Freeze Tolerance

To assess cold tolerance, stage-4 genotypes were subjected to freezing temperatures in two field experiments established at the Hague Farm of the Institute of Food and Agricultural Sciences, University of Florida, Hague, near Gainesville, FL. In two tests, CP 04-1566, 17 other stage-4 genotypes, and 3 reference cultivars (CP 72-2086, CP 78-1628, and CP 89-2143) were planted in the field on 24–25 Feb. 2009 and 26 Oct. 2010, both in an RCBD with four replications in single-row plots 1.5 m long and 2.4 m apart with 2.4-m breaks between replications. In the 2009 planted test, samples of five mature stalks were cut from each plot on 10 and 17 Dec. 2009 and on 10 and 27 Jan. 2010 in the first-ratoon crop. In the 2010 planted test, samples were similarly collected on 10 and 17 Dec. 2010 and on 10 and 27 Jan. 2011 in the first-ratoon crop and on 9 and 30 Nov. 2011, on 6 and 25 Jan. 2012, and on 9 Feb. 2012. Plots were exposed to temperatures between −3.0 and −6.0°C for 20 h before 10 Dec. 2010 and for an additional 20 h before 17 Dec. 2010. Later, plots were exposed to 35 h of temperatures between −3.0 and −7.5°C before 10 Jan. 2011, followed by 18 h of temperatures between −3.0 and −6.5°C before 27 Jan. 2011. Samples were returned to Canal Point for milling and analysis of sucrose content from extracted juice. Freeze-tolerance rankings were based on temporal deterioration of the percentage sucrose after exposure to freezing temperatures. A ranking of 1 signified the best freeze tolerance and a ranking of 21 signified the worst freeze tolerance; CP 04-1566 ranked 9th. CP 72-2086, CP 78-1628, and CP 89-2143 ranked 19th, 16th, and 11th

Table 5. Disease reactions of sugarcane cultivar CP 04-1566 and reference cultivars CP 72-2086, CP 78-1628, and CP 89-2143 in Florida.

Cultivar	Mosaic	Smut	Brown rust	Orange rust	Leaf Scald	Ratoon stunt	*Sugarcane yellow leaf virus*
CP 04-1566	MR†	MR	MR	MR	R	MR	S
CP 72-2086	S	R	MR	S	R	R	S
CP 78-1628	R	S	S	MS	MS	MS	S
CP 89-2143	MS	R	R	S	MS	MS	S

†R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

in freeze tolerance, respectively, in the 2009 planted test, and in the 2010 planted test, CP 04-1566 ranked 19th. CP 72-2086, CP 78-1628, and CP 89-2143 ranked 18th, 9th, and 7th in freeze tolerance, respectively

Availability

In its initial year of release, stalk sections for planting (seed cane) of CP 04-1566 will be available from the Florida Sugar Cane League, Inc. for commercial planting in Florida. It is not anticipated that patent protection for CP 04-1566 will be sought. Small quantities of seed cane for research purposes may be obtained at the USDA-ARS Sugarcane Field Station, Canal Point, FL where CP 04-1566 will be maintained for at least 5 yr from the date of this publication.

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